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Regulatory B cells, the key regulator to induce immune tolerance in organ transplantation

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In solid organ transplantation, especially renal transplantation, for the induction of immune tolerance, accumulating evidence has revealed that Regulatory B cells (Breg) play a crucial role in stimulating immune tolerance, alleviating immune responses, and improving graft survival. We describe the heterogeneous nature of Bregs, focusing on their defining surface markers and regulatory functions. Meanwhile, the major cytokine secretion function and the correlation between Breg and Treg or other immune checkpoints to balance the immune responses are addressed. Furthermore, we summarized the intrinsic and extrinsic pathways or costimulatory stimuli for the differentiation from naïve B cells. More importantly, we summarized the progression of the immune tolerance induction role of Breg in solid organ (kidney, liver, heart, lung, and islet) transplantation. This is an up-to-date review from the origin of Breg to the function of Breg in solid organ transplantation and how it induces immune tolerance in both murine models and human solid organ transplantation.

KEYWORDS

regulatory B cells (Breg), interleukin-10 (IL-10), solid organ transplantation, chemokines, B cell differentiation, immune tolerance

1 Introduction

In organ transplantation, the recipient's immune system has three major responses against the grafts (1), which are acute rejection, chronic rejection, and graft-versus-host disease (GVHD) (2). These immune responses are primarily initiated by T cell-mediated rejection, where donor antigens (particularly major histocompatibility complex (MHC) molecules) on the graft are recognized by the recipient's immune cells. For acute rejection, T cells and antibodies recognize and attack the graft within days or weeks after transplantation (3). Whereas chronic rejection is a prolonged and ongoing immune response that leads to gradual loss of graft function over time (4). These two immune responses are mostly in solid organ transplantation, and the immune response is the rejection of grafts, called host-versus-graft disease (HVGD) (5). However, for bone marrow and hematopoietic stem cell transplantation, there will be GVHD, a complication where donor immune cells attack the recipient's tissue (6). Therefore, to prevent these immune responses, immune tolerance to the graft has been investigated and established for years, whether in allogenic or xenogeneic transplantation.

For the induction of immune tolerance, accumulating evidence has revealed that Regulatory B cells (Breg) play a crucial role in stimulating immune tolerance, alleviating immune responses, and improving graft survival (7, 8). Bregs are a subset of B cells identified as having an immunosuppressive function, modulating the immune system to prevent excessive inflammation and autoimmune diseases (9, 10). Contrary to the role of B cells, which have traditionally been associated with antibody production and antigen presentation, Breg has been documented to contribute to immune homeostasis by regulating T cell responses (11) and anti-inflammatory cytokine production (12). Therefore, in this review, we comprehensively summarize the specific role of Breg in organ transplantation, including solid organ transplantation and hematopoietic transplantation.

2 Characteristics of Breg

Breg are a subset of B cells, which are not a uniform population, but rather a diverse group of cells (13). To date, there is no single marker universally accepted to identify all Breg, but several characteristics can define this subset of cells. The first one is $CD19^{+}CD25^{+}CD1d^{+}$ cells (14–16). These cell surface markers are often associated with regulatory B cells, even though they are not specific to Breg only. The second one is $CD24^{hi}CD38^{hi}$ cells (17–20). The third one is $IL10^{+}$ cells (21, 22). There is a discrepancy in $CD39^{hi}$ (23, 24) and $CD39^{-}$ (25) cells are Breg, therefore, CD39 might not be a canonical marker for Breg. The fourth one is IL-35 secreting B cells. This subset of B cells has been shown to suppress autoimmune diseases (26, 27), including autoimmune diabetes (28), systemic lupus erythematosus (29), ankylosing spondylitis (30), thyroid associated opthalmopathy (31) and also CNS (central nervous system) autoimmune disease (multiple sclerosis and uveitis infection) (32) as chronic hepatitis B (33). Further, IL-35-producing Breg could suppress inflammation and alveolar bone resorption in ligature-induced periodontitis (11). There is another subset of B cells that produce granzyme B, identified with $CD307b^{hi}$, $CD258^{hi}CD72^{hi}$, and $CD21^{lo}PD\text{-}1^{hi}$ B cell subpopulations (34). There are other subtypes of Breg, detailed addressed in Table 1.

The main immune regulatory function of Breg is realized by producing anti-inflammatory cytokines, interleukin-10 (IL-10) (35, 36), IL-35 (26, 27), IDO (37) and granzyme B (38, 39). As an anti-inflammatory cytokine, IL-10 is known for its immune-regulatory properties, such as inhibiting the activation of T cells (40), dendritic cells (41), and macrophages (42), and suppressing the production of pro-inflammatory cytokines (43). IL-10 can suppress inflammatory responses and the activation of immune cells, thereby regulating the inflammatory immune response. It primarily works by inhibiting the migration, infiltration, proliferation, and activation of inflammatory cells (44), and by suppressing the production of pro-inflammatory factors by Th1 cells (45, 46), thus inhibiting the cellular immune response (22). In addition, IL-10 can inhibit activated monocytes from secreting interleukin-1 (IL-1) and interleukin-6 (IL-6) (47), suppress the release of TNF-α by macrophages (48), and inhibit the activation of mast cells and the secretion of their cytokines (49, 50), thereby participating in the regulation of allergic reactions (40, 51). IL-10 also has an activating effect on B cells and can promote antibody production (52).

For the IL-35-producing Breg, they could be induced by IL-12p35 (53). Meanwhile, the production of IL-35 by Breg is facilitated through the binding of the BATF-IRF-4-IRF-8 complex to the promoter elements of the il12a and ebi3 genes (54). In the lung tissue of OVA-induced asthmatic mice, IL-35 enhances the presence of Breg that co-express IL-35 and IL-10, as well as conventional $LAG3^{+}$ regulatory T cells (55).

Breg also produces granzyme B. In liver transplant recipients with acute rejection, $CD19^{+}$ granzyme B-producing Breg serves as a feedback loop to modulate the activation of CD4+CD25- T cells (56). Due to the significance of granzyme B-producing Breg, S. Brouard laboratory generated a novel protocol to expand this subtype of Breg (39). The dysfunction of granzyme B-producing Breg is associated with more severe rheumatoid arthritis (38). Meanwhile, human granzyme B-producing Breg could inhibit the proliferation of effector $CD4^{+}CD25^{-}$ T effector cells (57).

3 The differentiation of Breg from naïve B cells

The differentiation of Breg from naïve B cells is via both intrinsic and extrinsic signals. B cells can be activated by the recognition of antigens through the B cell receptor (BCR) and also co-stimulatory signals (58). During this process, the BCR recognizes a specific antigen, typically in the form of proteins or polysaccharides on the surface of pathogens, dead cells, or other stimuli (59). Then, this antigen binds to the BCR to initiate intracellular signaling through the spleen tyrosine kinase (Syk) pathway (60) and subsequently activates downstream PLCγ2, PI3K, MAPK pathways (61, 62). With the activation of these

TABLE 1 Breg characteristics and their specific functions.

Subsets of Bregs	Transplantation or other disease	Function	References
CD19+CD25+CD1d+ cells	Renal transplantation	positively correlated with better graft function and longer and higher Treg level	(14)
CD19+CD24hiCD38hi cells	Renal transplantation	long-term graft survival of renal transplantation	(126)
	Renal transplantation	long-lasting graft survival of renal transplantation with drug-free	(128)
	Renal transplantation	longer survival with belatacep after renal transplantation	(131)
	Lung transplantation	Long-term lung grafts survival	(20)
CD19+CD24hiCD27+ Breg	Liver transplantation	predict the occurrence of acute allograft rejection in liver transplantation	(134)
CD19+CD5+CD1dhi Breg	Heart transplantation	Protective role in heart transplantation	(136)
	Islet transplantation	Responsible for the early stage of transplantation tolerance induction	(16)
CD39hi	Induce cytokine secretion	Induce IL-10 secretion	(23, 24)
CD39-	Breast cancer	Limited Th proliferation, type-1 cytokine production, and Teff survival. Stimulate Treg	(25)
B220+/Tim1+ Breg	Islet transplantation	Induce islet transplantation tolerance	(121)
CD19+TIM-1+Breg	Islet transplantation	Critical in the whole process of tolerance induction and maintenance	(16)
IL10+ B cells	Renal transplantation	Mouse model of renal transplantation, modulating T cell responses	(73, 124)
	Renal transplantation	Alleviate acute rejection of renal transplantation	(125)
	Renal transplantation	Alleviate renal injury after transplantation	(95)
	Renal transplantation	Prolong graft survival by decreasing CD3+ T cell proliferation	(127)
	Renal transplantation	Non-immunosuppressant for at least 1 year after renal transplantation	(129)
TGF-β-producing B cells	Renal transplantation	Tolerant drug-free patients with drug free	(130)
	Islet transplantation	Establish islet transplantation tolerance	(73)
IL-35-producing B cells	Autoimmune disease	Autoimmune diabetes	(28)
	Autoimmune disease	Systemic lupus erythematosus	(29)
	Autoimmune disease	Ankylosing spondylitis	(30)
	Autoimmune disease	Thyroid associated opthalmopathy	(31)
	Autoimmune disease	Multiple sclerosis and uveitis infection	(32)
	Autoimmune disease	Chronic hepatitis B	(33)
	Autoimmune disease	Periodontitis	(11)
granzyme B-producing B cells	Renal transplantation	Maintain allo-specific tolerance	(132)

signaling pathways, B cells are activated, proliferate, and survive. Beyond antigen recognition, Breg require co-stimulatory signals for full activation (23, 63). CD40 signaling is a crucial co-stimulatory pathway that activates B cells (64). CD40 ligation by CD40L (present on T cell surface) triggers several downstream signaling events that influence the differentiation program of the B cell, including NF-κB activation, which is crucial for B cell survival and activation (65–67). Several transcription factors are involved in guiding naïve B cells to adopt a regulatory phenotype, and they form part of the intrinsic genetic pathway for Breg differentiation. Several transcription factors contribute to the development of IL-10-producing Breg, including Bach2 (68), BRD4 (69), the Nuclear Factor Kappa-B (NF-κB) signaling pathway (70), Interferon Regulatory Factor (IRF4) (54), STAT3 and c-MAF (71), Foxp3 (72), Transforming Growth Factor-beta (TGF-β) signaling (73, 74), IL-21 (75, 76) (a cytokine produced by T helper type 17 (Th17) and follicular helper T (Tfh) cells), and B Lymphocyte-Induced Maturation Protein 1 (BLIMP-1) (77). Furthermore, toll-like receptors (TLRs) are crucial for the induction of IL-10-producing Breg (78). By antigen-presenting cells (APCs), signals from TLRs are essential for IL-10 production. Autophagosomes released by tumors stimulate the formation of IL-10-producing Breg, which in turn suppress T lymphocyte activity through the TLR2-MyD88-NF-κB signaling pathway (79). For the generation of granzyme B-

producing Breg, it could be generated by B-chronic lymphocytic leukemia (B-CLL) cells treated with interleukin-21 (IL-21) (80). $CD4^+$ T cells can produce IL-21 and rapidly induce granzyme B-producing Breg in co-cultured B cells in an IL-21 receptor-dependent manner (81, 82). Lymphotoxin alpha, a new and potent Breg ligand, has also been reported to increase granzyme B expression in Breg (57).

The extrinsic pathway refers to the external signals from the microenvironment, such as cytokines, cellular interactions, and immune stimuli, that influence the differentiation of naïve B cells into Breg. These extrinsic signals include immune cells (T cells, dendritic cells, and macrophages) (83–86), cytokines produced during inflammation or tolerance induction, and tissue-specific microenvironments (23, 87). For the cytokine-driven Breg differentiation it includes IL-10, TGF-β, and IL-21, along with interactions with T cells, dendritic cells, and other immune cells, providing additional stimuli for the differentiation of Breg. Moreover, Indoleamine 2, 3-dioxygenase (IDO) could be generated by Breg (37), and in turn, it could induce Breg infiltration in lung cancer (88). Mesenchymal stromal cells alleviate multiple sclerosis by increasing the suppressive proportion of $CD5^+$ $IL\text{-}10^+$ Breg in an IDO-dependent manner (55).

4 The function of the Breg

The primary function of the Breg is to regulate immune responses and maintain tolerance (73). They achieve this primarily through the production of immunosuppressive cytokines like IL-10, as well as through other mechanisms.

The most important function of Breg is the suppression of T cell responses (84, 89). Breg play a crucial role in controlling T cell activity, especially $CD4^+$ T helper (Th) cells (90–92). By producing IL-10, they inhibit the activation, proliferation, and cytokine production of effector T cells (Teff), thus preventing excessive immune responses and alleviating tissue damage (83, 85). They can also induce the differentiation of regulatory T cells (Tregs), which further enhances immune suppression (83, 91, 93). Meanwhile, Breg could regulate the inflammatory cytokines by suppressing the production of pro-inflammatory cytokines, such as TNF-α (94, 95), IL-6 (96), IL-17 (97, 98), and IFNγ (99).

5 Mechanisms of Breg in organ transplantation

Breg contribute to immune tolerance in organ transplantation through a variety of mechanisms, many of which revolve around their capacity to suppress excessive immune responses, inhibit T-cell activation, and promote an anti-inflammatory microenvironment (8, 100, 101). The main mechanisms are as follows:

5.1 Bregs mediate immune tolerance via anti-inflammatory cytokines

The primary function of Breg in transplantation is the secretion of anti-inflammatory cytokines, particularly IL-10. IL-10 suppresses immune responses in several ways. It could inhibit T-cell activation (102, 103). IL-10 directly suppresses the activation, proliferation, and cytokine production of effector T cells (both $CD4^+$ and $CD8^+$ T cells), preventing them from recognizing and attacking the transplanted organ (104, 105). Besides, it could stimulate the differentiation and expansion of regulatory T cells (Tregs) by IL-10 (106). Tregs, in turn, regulate both T cell-mediated and antibody-mediated rejection (107). Further, it could inhibit the antigen-presenting cells (APCs) (108). IL-10 downregulates the activity of APCs such as dendritic cells, macrophages, and B cells, reducing their capacity to stimulate T cell responses.

5.2 Regulation of B cell responses to reduce graft rejection

Breg also exert their immunosuppressive effects on B cells by modulating the activation and function of other B cell subsets. In transplantation, the role of B cells can be complex, as they contribute to both humoral rejection (antibody-mediated rejection) and immune regulation (109). In transplantation, Breg help suppress the activation of autoreactive B cells that produce antibodies against the graft (110). By reducing the production of alloantibodies (antibodies that recognize donor antigens), Breg help prevent antibody-mediated rejection (111). Furthermore, it controls antigen-specific B cell responses. Breg can inhibit the expansion and differentiation of antigen-specific B cells that produce graft-specific antibodies, which could otherwise contribute to graft rejection. To induce allograft tolerance, Bregs can be induced by anti-CD45RB and anti-TIM1antibody, which means that Breg requires antigen recognition for tolerance inducition (112).

5.3 Direct suppression of T cell-mediated rejection

In transplantation, Breg can directly suppress T cell responses via cell-to-cell contact, in addition to cytokine secretion. The first way is to induce immune checkpoint proteins. Breg expresses inhibitory molecules like PD-L1 (113–115), CTLA-4 (84), and TIGIT (116), which can interact with their respective ligands on T cells to induce immune suppression. These interactions inhibit T-cell activation and promote tolerance. The second way is via the induction of anergy in T cells. Breg can induce anergy in $CD4^+$ and $CD8^+$ T cells through direct interactions, preventing them from responding to allo-antigens (117). The third way is via the induction of Treg differentiation. Through direct contact, Breg can promote

the conversion of naïve T cells into regulatory T cells (Tregs), which are crucial for maintaining immune tolerance to the graft (91).

5.4 Induction of graft-specific tolerance

Breg is involved in the establishment and maintenance of graft-specific tolerance, which is essential for long-term organ survival without the need for chronic immunosuppression (2). Breg could induce donor-specific tolerance by promoting the tolerance specifically to donor antigens. This may involve the promotion of Tregs or other regulatory cells that target graft-specific immune responses, allowing the recipient's immune system to accept the transplanted organ as "self" (2). Furthermore, Breg could regulate inflammatory responses in the transplant microenvironment (87, 118). Breg helps to create an anti-inflammatory environment within the graft, which reduces the activation of both innate and adaptive immune responses that could lead to graft rejection (7, 8, 101, 119).

5.5 Interactions between Bregs and other immune cells in transplantation

By interacting with other immune cells, Breg could stimulate tolerance in transplantation. Breg can modulate dendritic cell (DC) function, reducing their ability to activate T cells. By interacting with DCs, Breg can decrease the presentation of donor antigens and thereby lower the risk of graft rejection (83). Breg could mitigate the function of macrophages, which play a central role in transplant rejection and immune surveillance (120). By reducing macrophage activation, Breg can help prevent tissue damage in the graft. For the natural killer (NK) cells, there is emerging evidence suggesting that Breg may also interact with NK cells, which are involved in innate immunity and can contribute to graft rejection. Breg can inhibit NK cell cytotoxicity and promote immune tolerance in the transplantation (121).

6 Breg in solid organ transplantation

6.1 Renal transplantation

In solid organ transplantation, Breg contributes to the induction of tolerance and the prevention of both acute and chronic rejection (8, 122, 123). Studies in mouse models of renal transplantation show that Breg play a role in the tolerance of the grafts by modulating T cell responses and promoting IL-10 production (73, 124). In a cohort of 200 kidney transplant recipients, an imbalance of circulating follicular helper T cells (cTfh) over IL10$^+$ Breg leads to graft failure. Meanwhile, the increase in the cTfh/IL10$^+$Breg ratio is an index of acute rejection (125). A cohort of human renal transplantation with calcineurin inhibitors (CNI) or mammalian target of rapamycin (mTOR) inhibitors showed that CD19$^+$CD24^{hi}CD38^{hi} Breg increases over time and contributes to the long-term graft survival is not correlated with these drugs (126). In human allogenic renal transplantation, Breg could regulate the IL-10 and TNF-α expression ratios to alleviate renal injury after transplantation (95). Moreover, in kidney transplant recipients, the levels of CD19$^+$CD25$^+$ Breg are positively correlated with better graft function and longer and higher Treg levels (14). In another study of human kidney allografts, human leukocyte antigen G (HLA-G) stimulates IL-10-producing memory Breg (CD19$^+$CD24^{hi}CD27$^+$IL-10$^+$) to prolong graft survival by decreasing CD3$^+$ T cell proliferation (127). Renal transplant recipients could benefit from the induction of long-lasting CD19$^+$CD24^{hi}CD38^{hi}Breg (128). In renal transplant recipients, a higher level of CD19$^+$CD25$^+$ Breg is independently associated with improved graft function (14). In a cohort of 58 kidney transplant recipients, IL-10-producing Breg could lead to non-immunosuppressant for at least 1 year after transplantation (129). Further, T1 and T2 transitional B cells (CD38$^+$CD24$^+$) were also increased in tolerant recipients. The 42 healthy controls also had IL-10-producing Breg. But they found no difference in TGF-β secreting B cells (129). In another cohort study with 71 kidney transplant recipients and 19 healthy controls, T1 and T2 transitional B cells (CD38$^+$CD24$^+$) were also increased in tolerant recipients, who had higher percentages of B cells and less NK and T cells. In the analysis of the tolerant drug-free patients, there is a redistribution of Breg, which produces TGF-β instead of IL-10 (130). In a Phase III clinical study of belatacept on kidney transplant recipients, the frequency and absolute number of transitional B cells, including CD19$^+$CD24^{hi}CD38^{hi} Breg and CD19$^+$IgD^{hi}CD38^{hi}CD27$^-$, and naïve B cells were significantly higher (131). Granzyme B-producing B cells are a characteristic B cell subset, identified with CD307b^{hi}, CD258^{hi}CD72^{hi}, and CD21^{lo}PD-1^{hi} B cell subpopulations (34). This subtype of Breg serves a dual function in renal transplantation. They act as regulatory cells to maintain allo-specific tolerance and as effector cells to enhance CMV viral control (132).

6.2 Other solid organ transplantation

Breg in liver transplant models has been shown to promote long-term graft survival by suppressing immune responses and promoting donor-specific tolerance (101). This is particularly important in liver transplantation, as the liver is considered to be an immunologically privileged organ, and Breg may help maintain this privilege (133). Meanwhile, the proportion of CD19$^+$CD24^{hi}CD27$^+$ Breg has been reported to predict the occurrence of acute allograft rejection in liver transplantation (134). With the application of Sirolimus, both Breg and Treg are expanded in liver transplant patients (135).

For heart and lung transplantation, similar to kidney and liver transplantation, Breg contributes to immune regulation and graft survival in heart and lung transplant models. In the heart transplantation mouse model, histone deacetylase (HDAC) inhibitor trichostatin A (TSA) could increase the frequency of IL-10 and TGF-β-producing CD19$^+$CD5$^+$CD1d^{hi} Breg cells and thereby induce immune tolerance (136). With the adoptive transfer of the transplanted Breg in heart-transplanted mice, this

Breg could induce transplantation tolerance via the CD40-TRAF6 signaling pathway in DCs (137). In a study of 117 cases of clinical lung transplantation recipients, $CD19^{+}CD24^{hi}CD38^{hi}$ B-cells contribute to the long-term lung grafts survival (20).

For allogenic islet transplantation, two subsets of Breg play a key role in tolerance induction and maintenance. $CD19^{+}CD5^{+}CD1d^{+}$ B10 cells are mainly responsible for the early stage of transplantation tolerance induction, and $CD19^{+}TIM\text{-}1^{+}$B cells are critical in the whole process of tolerance induction and maintenance (16). Another study on islet transplant tolerance revealed that Breg-dependent tolerance is dependent on NK cells (121). In a mismatched islet transplantation model, to establish transplantation tolerance, adoptively transferred Breg cells need the presence of Treg (73).

7 Conclusion and future perspective

Breg in immune regulation as Tregs, especially in organ transplantation, offers significant therapeutic potential and provides promising potential for Breg-based therapies (138). Enhancing the function or expansion of Breg could be a promising therapeutic strategy to induce tolerance and promote graft survival (8, 139). It could be a substitute for immunosuppressive drugs, which may have significant adverse effects (140) (Figure 1).

Despite the promising role of Breg in organ transplantation, several challenges remain. One major unresolved issue is the heterogeneity of Breg subsets and the lack of standardized markers for their identification, making their clinical translation challenging. Additionally, while Breg-based therapies hold potential for inducing long-term tolerance, concerns remain regarding their stability, potential off-target effects, and the risk of over-suppressing the immune system. The optimal strategies for *in vivo* expansion or adoptive transfer of Breg also require further refinement. Future research should focus on defining the molecular mechanisms governing Breg differentiation and function, optimizing methods for their therapeutic application, and conducting long-term clinical studies to evaluate their efficacy in transplantation. Integrating Breg-based therapies with current immunosuppressive strategies

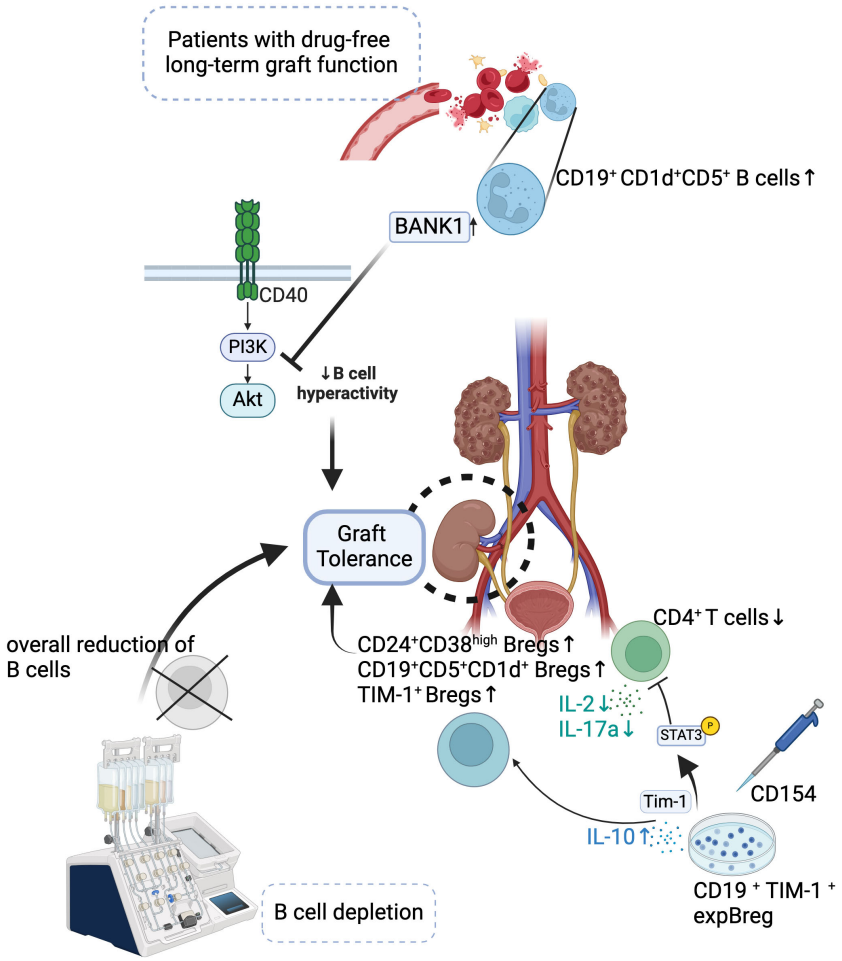

FIGURE 1
Mechanisms of Bregs in Promoting Graft Tolerance. Bregs contribute to long-term drug-free graft tolerance in transplant patients. Increased populations of $CD24^{+}CD38hi$ Bregs, $CD19^{+}CD1d^{+}CD5^{+}$ B cells, and $TIM\text{-}1^{+}$ Bregs, along with reduced $CD4^{+}$ T cells, promote graft tolerance. BANK1-mediated inhibition of PI3K-Akt signaling reduces B cell hyperactivity, enhancing tolerance. Approaches like B cell depletion or expansion of $TIM\text{-}1^{+}$ Bregs (expBregs) further support graft survival.

may offer a novel approach to reducing drug toxicity while maintaining immune tolerance. Addressing these challenges will be crucial for advancing Breg-based immunotherapy in organ transplantation.

Overall, Breg plays a critical role in maintaining immune tolerance and promoting graft survival in organ transplantation. The therapeutic potential of Breg provides new hope for cell therapy in organ transplantation.

Author contributions

JinL: Writing – original draft. YY: Writing – original draft. JisL: Writing – original draft. ZL: Writing – original draft. SS: Conceptualization, Investigation, Writing – review & editing. YZ: Conceptualization, Funding acquisition, Investigation, Writing – review & editing. YW: Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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